THE ACTIVE PRINCIPLE OF A BINI SPEAR POISON

BY

P. P. LAIDLAW, M.A., B.C.

(*Reprinted from the "Journal of Physiology,"* Vol. xxxix, No. 5, December 23, 1909)

From
THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
BROCKWELL HALL
HERNE HILL
LONDON, S.E.

[*Reprinted from the Journal of Physiology,*
Vol. XXXXI. No. 5, *December* 23, 1909.]

THE ACTIVE PRINCIPLE OF A BINI SPEAR POISON.

By P. P. LAIDLAW.

(*From The Wellcome Physiological Research Laboratories, Herne Hill, S.E.*)

Through the kindness of S. Sproston, Esq., I have been able to examine two spear heads poisoned for elephant hunting, which were obtained from West Africa. They were obtained by him from a Bini huntsman of Benin City, who is the only successful maker of the poison in the district. The composition and method of preparation of this poison is a secret handed down from father to son. The huntsman would not disclose the actual ingredients of the poison, but stated that vegetable matter only was used in its manufacture, that it took two or three months to prepare, and that it retained its activity for years. The use of poisoned spears and arrows in elephant hunting has been recorded before, but owing to the large size of the animal and the small dose of the poison introduced by a spear or arrow wound there is usually a long time interval ("half a day") between the wound and the death of the quarry.

The Niger District is quoted by Fraser[1] among eight others as a locality in which the natives use strophanthus as an arrow poison. The Munchi[2] arrow poison also comes from this district.

The two iron spear heads were thickly coated all over with a dark brownish green coloured mass of glue-like consistency. An extract in ·6 % salt solution was made and injected into an intact frog. At the end of half an hour no sign of heart beat could be detected, and reflexes were very feeble. In a few minutes more no response could be elicited to mechanical stimulation. Post-mortem the ventricle was pale and firmly contracted, the auricles engorged with blood; central and peripheral electrical stimulation of the cut sciatic nerve evoked no response

[1] Fraser. *Trans. Roy. Soc. Edin.* xxxv. p. 955. 1889.

[2] Cf. Fröhlich. This *Journal*, xxxii. p. 319. 1905. Also Mines. *Ibid.* xxxvii. p. 37. 1908.

in the muscles though they responded well to direct stimulation. A 1 % extract of the poison in ·6 % saline was found to be very active. ·05 c.c. of such an extract killed a 30 gram frog in the course of two hours. The ventricle was arrested in systole. These experiments were

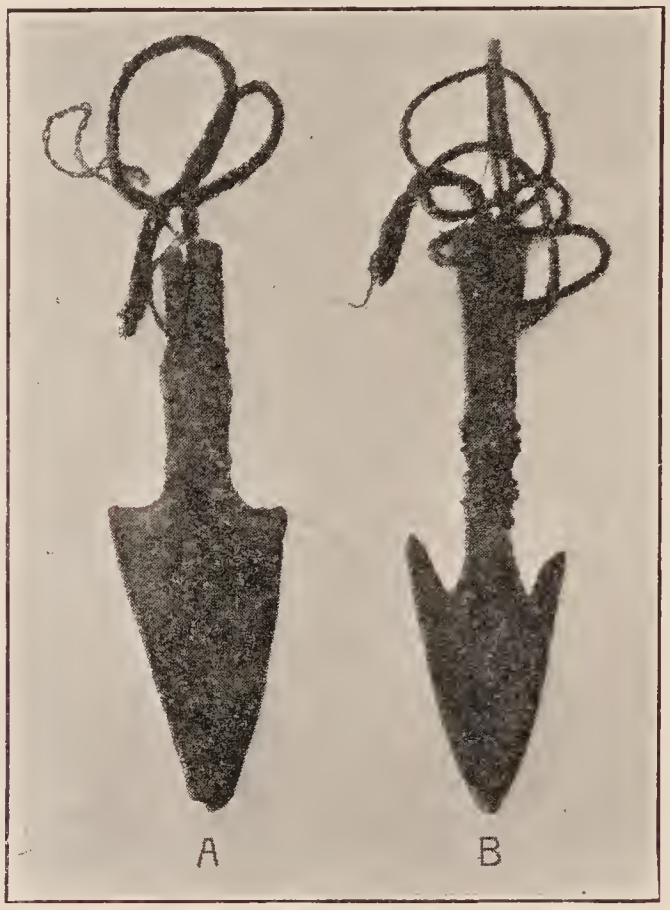

Fig. 1. Appearance of spear heads
A. Before removal of poison,
B. After removal of poison.
Greatest length $5\frac{1}{4}$ inches, greatest breadth $1\frac{1}{2}$ inches.

repeated with similar results—systolic arrest of the ventricle, in some instances a curare-like effect on motor terminations, and a feeble or absent response to central stimulation of the cut sciatic.

A solution of the poison in saline lost activity on hydrolysis with hydrochloric acid. $\frac{1}{2}$ c.c. and 1 c.c. of a dilute solution of the poison proved fatal to two frogs in an hour and a half. This solution was hydrolysed with hydrochloric acid, neutralised, brought back to original volume. $\frac{1}{2}$ c.c., 1 c.c. and $1\frac{1}{2}$ c.c. injected into frogs had no perceptible effect, and two days afterwards the frogs were alive and appeared normal.

A poison with such high toxicity and characteristic physiological action, is probably strophanthin. Recognised methods for the isolation of strophanthin were therefore employed. The poison was scraped off the spear heads after a preliminary soaking in 70 % alcohol. It was then

ground up in a mortar with a little alcohol and extracted twice with warm 70 % alcohol. The solid left behind was only slightly active. It contained some hard gritty particles, but for the most part consisted of disintegrated vegetable matter (microscope).

The alcoholic extract was very active. It was evaporated down to small bulk when some dark brown oily drops separated. Some water was added and the whole precipitated with basic lead acetate. The lead precipitate carried down all the finely divided oil drops and left a clear yellow solution. The excess of lead was removed by sulphuretted hydrogen, the filtrate taken to a small bulk and set aside in a desiccator in the hope that the strophanthin would crystallise out (Feist[1], Kohn and Kulisch[2]). The solution became a thick gum. This being unsuccessful the gummy mass was dissolved in water and saturated with ammonium sulphate (Thoms[3]): a slimy precipitate separated which was taken up by amyl alcohol. The residue after evaporating off the amyl alcohol was taken up in alcohol and attempts at crystallisation were made from water, alcohol, methyl alcohol, acetone, all of which freely dissolved the product, but all attempts failed. The product was insoluble in chloroform, ether, acetic ether, and petroleum ether. Precipitation with these did not appear to purify the product further. It has a bitter taste, and when finely powdered has an irritating effect on the mucous membrane of the nose. It gives a fine green colour with strong sulphuric acid which becomes a brown green on standing.

On hydrolysis it yields a reducing sugar from which however no crystalline osazone was obtained (see Feist, *loc. cit.*).

The product was tested on frogs, cats and rabbits. In all cases the mode of death was by arrest of the heart (ventricle in systole). The minimum lethal dose was determined for frogs using a three hour limit: 0·12 mgrm. per 100 grms. killed within the limit and 0·1 mgm. per 100 outside the limit. The minimum lethal dose of strophanthin from strophanthus gratus (the most potent of the strophanthins) per 100 grm. frog is ·07—·08 mgrms[4].

Three or four mgrms. of the product, when injected into a cat produced a rise of blood-pressure and some slowing of the heart beat, a little later the heart became very rapid and irregular, the blood-pressure rose still further and showed undulations; ultimately it

[1] Feist. *Chem. Centralb.* I. p. 946. 1898.
[2] Kohn and Kulisch. *Ibid.*
[3] Thoms. *Ber. d. Deut. pharmaceut. Gesellsch.* p. 104. 1904.
[4] Ziegenbein. *Ber. d. pharm. Gesellch.* XII. p. 335. 1902.

somewhat suddenly fell to zero. Post-mortem the ventricles were found firmly contracted.

Isolated rabbits' hearts perfused with Ringer's solution using the Locke technique, showed augmentation of beat when the drug was perfused through the heart in a concentration of 1 in 200,000. A little later delirious irregularity set in and the heart stopped in systole. Fig. 2 shows the several stages in such an experiment.

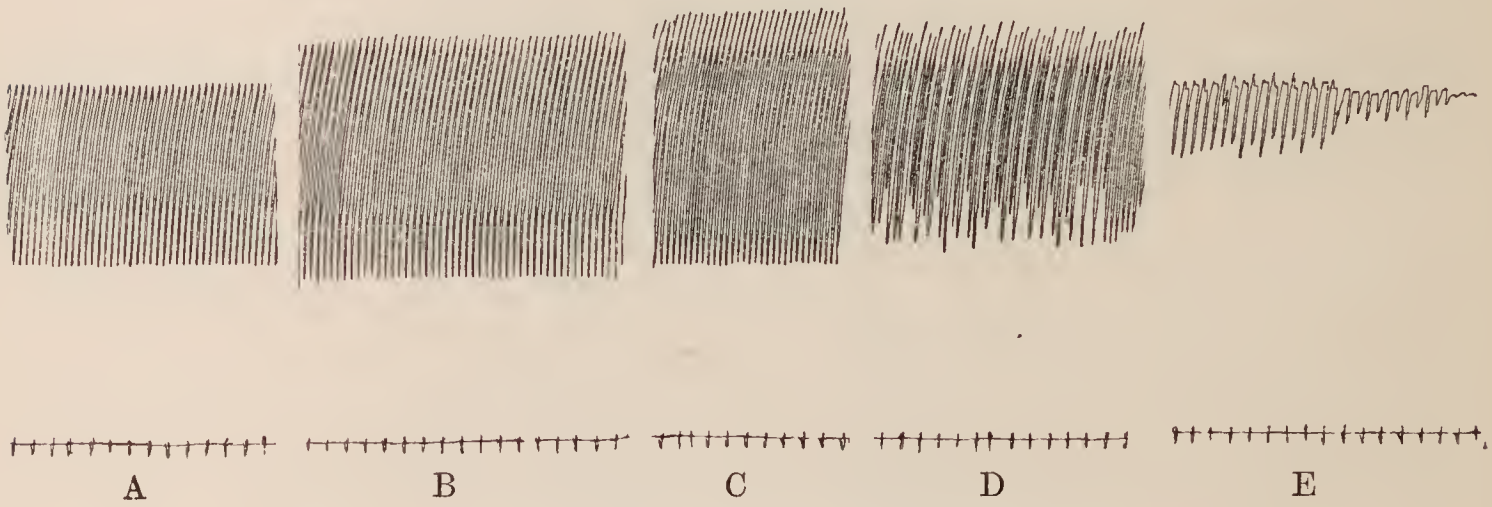

Fig. 2. Isolated rabbit's heart, Locke-Langendorf method.

A. Normal.
B. After 50″ perfusion with 1 in 200,000 crude strophanthin from spear poison.
C. 2 minutes later.
D. 70″ later.
E. 70″ later.
Upstroke = systole, time = seconds.

It seems clear that the active principle of the poison belongs to the class of glucosides, gives the characteristic colour reaction of Kombé strophanthin, and possesses the solubilities and physiological properties of that substance.

The rather high minimum lethal dose for the purest product obtained, is quite consistent with this view since certain impurities must have been present which prevented crystallisation. Two white substances were isolated from the poison, which crystallised in needles. As they were inactive when tested physiologically they were discarded.

Attempts to make crystalline strophanthidin from the remainder of the product failed.